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A polymer encapsulation approach to prepare zwitterion-like, biocompatible quantum dots with wide pH and ionic stability

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Abstract

A surface modification approach adopting polymer encapsulation was developed to prepare zwitterion-like quantum dots (ZWL-QDs). The fundamental physical, chemical, and biological properties of the ZWL-QDs were characterized. It is found that the ZWL-QDs almost preserve the

quantum yield (QY) of native hydrophobic QDs in organic solvents, and also are compact in size (7 ~ 10 nm hydrodynamic diameter) and stable over wide pHs or in high salinity solutions. Further cellular study shows that the ZWL-QDs with a concentration less than 100 nM have a minimal cytotoxicity and thus are biocompatible. Characterizing and understanding these essential properties of the ZWL-QDs are an important step before employing them for various applications.

Keywords

Nanoparticle surface modification; Polymer encapsulation; CuInS₂/ZnS quantum dots; Zwitterionic like; Colloidal stability

Introduction

High-quality quantum dots (QDs) with high quantum yield (QY) and narrow size distribution are usually synthesized at high temperature in organic solvents, and thus capped with hydrophobic ligands such as trioctylphosphine oxide, oleylamine, or dodecanethiol. QDs capped with these ligands are not soluble in aqueous solutions. For their use in biological or biomedical applications, further surface modification of these QDs is required to make them biocompatible and water soluble (Bruchez et al. 1998; Resch-Genger et al. 2008).

Ligand exchange and polymer encapsulation are two common surface modification technologies (Bruchez et al. 1998; Resch-Genger et al. 2008; Sperling and Parak 2010). Ligand exchange adopts hydrophilic ligands to replace hydrophobic ligands on QD surfaces. The hydrophilic ligands necessarily have a terminal binding group (e.g., thiol) with a high affinity for QD surface atoms. In the polymer encapsulation approach, amphiphilic polymers are often used. The hydrophobic portion of amphiphilic polymer intercalates with the hydrophobic ligands on QD surface, while exposing the hydrophilic portion to aqueous solutions. To further enhance QD colloidal stability and biocompatibility, polyethylene glycol (PEG) chains (due to their good steric stabilization capability) are usually incorporated into hydrophilic ligands or amphiphilic polymers (Uyeda et al. 2005; Zimmer et al. 2006; Susumu et al. 2007; Yu et al. 2007; Mei et al. 2008; Lees et al. 2009; Delehanty et al. 2013). However, PEG substantially increases QD size and restricts access to confined spaces. PEG QDs also tend to aggregate in high salinity buffers (Muro et al. 2010).

Zwitterionic groups present an alternative solution to PEG in preparing more compact nanoparticles with high stability in harsh conditions (wide pH ranges or high salt concentrations) (Breus et al. 2009; Muro et al. 2010; Muro et al. 2012; Liu et al. 2013). Once zwitterionic groups such as carboxybetaine (CB) and sulfobetaine (SB) are coated on QD surfaces to form zwitterionic QDs (ZW-QDs), the positive and negative electrical charges in zwitterionic groups can interact with water molecules to form a hydration layer and thus make QDs less susceptible to the ionic strength and pH changes. To prepare ZW-QDs, zwitterionic groups are usually coupled to thiol ligands such as dihydrolipoic acid (DHLA), where these coupled ligands exchanged with hydrophobic ligands on QD surfaces. Although these ZW-QDs are compact and stable, the synthesis of the coupled ligands involves many complicated and costly steps. Moreover, these QDs suffer intrinsic

drawbacks of ligand exchange (Susumu et al. 2007; Resch-Genger et al. 2008; Mei et al. 2008). First, ligand exchange alters the chemical and physical states of the QD surface atoms and thus significantly lowers the QY. Second, thiols are apt to oxidize in the presence of oxygen and therefore lose their binding affinity to deteriorate QD stability. On the other hand, polymer encapsulation does not involve the drawbacks of ligand exchange and thus are of interests for the preparation of ZW-QDs or zwitterion-like (ZWL) QDs (Yezhelyev et al. 2008; Bagalkot and Gao 2011; Booth et al. 2013). In this study, considering the incorporation of positive/negative charges in amphiphilic polymer to form zwitterion-like quantum dots (ZWL-QDs) is feasible, a surface modification method adopting the charge incorporation strategy as well as polymer encapsulation has been developed to prepare ZWL-QDs.

As shown in Fig. 1, poly (maleic anhydride-alt-1-octadecene) (PMAO) (Sigma-Aldrich) was coupled with 3-(dimethylamino)-1-propylamine (DMAPA) first. In the second step, the resultant PMAO-amine polymers were further mixed with QDs to allow the formation of hydrophobic interactions between the native ligands of QDs and the long alkane chain of the polymer. Methyl iodide (CH_3I or MeI) was then added to quaternize the tertiary amino groups on the polymer. This surface has positive and negative charges for colloidal stability in solutions with a wide range of pH and high salinity, and a minimal hydrodynamic diameter by avoiding PEG chains. Since the original hydrophobic ligands on QD surfaces remain during the surface modification, the QY of ZWL-QDs is almost same as that of hydrophobic QDs in organic solvents. Compared to ligand exchange using DHLA-CB or DHLA-SB, this two-step approach is simple and cost effective, and avoids the shortcomings of thiols.

In addition, some recent studies have reported that positive charges on nanoparticle surface can be used to absorb negative molecules such as small interference RNAs for drug delivery into cells or tissues (Yezhelyev et al. 2008; Kievit et al. 2009; Giljohann et al. 2009; Veisheh et al. 2010; Lee et al. 2010; Bagalkot and Gao 2011). In this context, ZWL-QDs also have the potential to be used as drug carriers or vehicles.

Experimental methods

Chemicals and apparatus

Copper (I) acetate (97 %), zinc acetate (99 %), oleic acid (90 %), oleylamine (70 %), 1-octadecene (ODE, 90 %), poly(maleic anhydride-alt-1-octadecene) (PMAO) (average Mn 30,000–50,000 as a powder), 3-(dimethylamino)-1-propylamine (DMAPA) (99 %), methyl iodide (99 %), poly-D-lysine, and gelatin were purchased from Sigma-Aldrich. Indium (III) acetate (99.99 %) and 1-dodecanethiol (DDT, 98 %) were purchased from Alfa Aesar. RPMI-1640 and MEM media were from Corning Cellgro. Heat-inactivated fetal bovine serum (FBS) was from Gibco. Fluorescein diacetate/propidium iodide (FDA/PI) was from Invitrogen. Dulbecco's phosphate-buffered saline (DPBS) was from Fisher Scientific. U-87 MG and HEK-293 cells were ordered from ATCC. Chemicals were used as received without further purification.

The ultraviolet and visible (UV–Vis) spectra of QDs in organic solvents and aqueous solutions were obtained with a UV–Vis spectrometer (UV-2450 from Shimadzu) using cuvettes with a path length of 1 cm. Photoluminescence spectra were acquired using a spectrophotometer (RF-5301PC from Shimadzu). Infrared (IR) spectra of polymers and QDs were collected using a FT-IR spectrometer (Perkin-Elmer Frontier) equipped with attenuated total reflectance and measurement software (Spectrum 10). Particle size measurements of QDs were made using a ZetaPALS dynamic light scattering detector (Brookhaven Instruments Corp.).

CuInS₂/ZnS QD preparation

For the preparation of CuInS₂ (CIS) nanocrystals, indium acetate (0.1 mmol), copper (I) acetate (0.1 mmol), DDT (2.0 mL), oleic acid (0.4 mL), and 6 mL of octadecene (ODE) were added into a 50 mL three-necked, round-bottom flask. The reaction solution was degassed under vacuum for 10 min at room temperature until no bubbles were observed. Solutions were then heated to 140 °C under vacuum using a heating mantle. An argon atmosphere was next introduced into the flask and the temperature of the resulting solution was quickly raised to 260 °C at a rate of 10 °C/min. The reaction lasted for 10 min and then the reaction flask was cooled down in room temperature.

To promote ZnS shell growth on CIS cores, Zn-precursor solution (0.4 M zinc acetate in ODE/oleylamine (4:1)) was injected to the core growth solution after the temperature of the reaction mixture lowered to 230 °C. Zn-precursor solution (4.5 mL of 0.4 M zinc acetate in ODE/oleylamine (4:1)) was injected into the NC suspension solution, drop by drop with a syringe over two hours. After the reactions were complete, mixtures were cooled down to room temperature in air and CIS/ZnS QDs were purified using hexane and ethanol.

PMAO-DMAPA polymer synthesis

Poly(maleic anhydride-alt-1-octadecene (PMAO) (1.28 g, average Mn 30,000–50,000, 114 anhydride groups, 3.65 mmol of anhydride) was weighed into a 100-mL round-bottom flask and 15 mL of dry chloroform (treated with Al₂O₃) was added to dissolve the PMAO completely. The resulting solution was then stirred for 10 min at room temperature and the solution was cooled down to ~5 °C using an ice bath. 3-(dimethylamino)-1-propylamine (DMAPA) (481 µL, 1.05 equivalents relative to the amount of anhydride on PMAO polymer) was added to the mixture. The resulting solution was stirred at ~5 °C for 3 h. Acetone (50 mL) was added to form a precipitate and the solution was allowed to settle down for 30 min. A white solid was collected on a glass filter using suction filtration and washed with excess acetone. The solid was then dried under vacuum overnight at room temperature to produce PMAO-DMAPA (1.40 g, 84 %).

Preparation of ZWL-QDs using polymer encapsulation

PMAO-DMAPA polymer (0.18 g) was added to 15 mL of chloroform in a 100-mL round-bottom flask and dissolved completely with the aid of sonication. CIS/ZnS QDs (15 mg in 1.5 mL chloroform) was added into the flask and the resulting solution was stirred at room temperature overnight. Methyl iodide was added and the resultant solution was stirred at room temperature for 2 h. Pure water (20 mL) was added to the chloroform-based solution

containing complexes. Chloroform was slowly removed using rotary evaporation at room temperature. A clear solution with a colored portion of water-soluble QDs was obtained, suggesting 100 % transfer of QDs from chloroform to water. To remove larger impurities, the solution was passed through a 0.2 μm Nylon syringe filter. ZWL-QDs were further purified using ultrafiltration and centrifugation.

Cell uptake and cytotoxicity study of ZWL-QDs

A U-87 MG human brain glioblastoma cell line (ATCC HTB-14) was cultured (37 °C, 5 % CO₂) on 24-well plastic plates coated with 0.1 % Gelatin in MEM (Corning Cellgro) medium with 10 % FBS (Gibco, heat-inactivated) overnight. The human embryonic kidney cell line HEK293 (ATCC CRL-1537) was cultured (37 °C, 5 % CO₂) on 24-well plastic plates (coated with Poly-D-lysine, Sigma) in RPMI-1640 (Corning Cellgro) medium with 10 % FBS (Gibco, heat-inactivated) overnight. The cells were incubated with 20 nM ZWL-QDs in growth medium for 4 and 16 h. Then, they were gently rinsed with DPBS before imaging. For the QDs cytotoxicity study, cells were incubated with ZWL-QDs in full growth medium at various concentrations. At different culture times (up to 72 h), cells were gently rinsed with DPBS, and then stained with FDA/PI to determine live versus dead cells. Dead cells (red spots in images) and live cells (green spots in images) were counted manually in sufficient numbers to attain statistical significance. The cell death rate was calculated as the ratio of dead cells to live cells, and the cell viability was calculated as 100 % minus cell death rate. The relative cell viability is the ratio of the cell viability of the sample to that of the control. All images were collected using a Life Technologies EVOS-FL microscope.

Results and discussion

During the preparation of ZWL-QDs, instead of cadmium-based QDs, more environmentally benign CuInS₂/ZnS QDs were used. Briefly, indium(III) acetate, copper(I) acetate, 1-dodecanethiol (DDT), oleic acid (OLA), and 1-octadecene (ODE) were heated to 260 °C in an argon environment to produce CuInS₂ cores. Zn-precursor solution was then injected into the core growth solution to produce ZnS shells on the CuInS₂ at 230 °C. The CuInS₂/ZnS QDs were grown to emit red fluorescence (~660 nm), and their optical properties and material characteristics have been reported previously (Huang et al. 2013). The original hydrophobic ligands on CuInS₂/ZnS QDs are DDT and OLA.

Figure 2 shows the FT-IR spectra of materials during each synthetic step of producing ZWL-QDs. Notably, the spectrum of PMAO-DMAPA shows a diminishing bands at 1780 cm⁻¹ but more bands appear between 1780 cm⁻¹ and 1500 cm⁻¹, compared to that of PMAO. These changes indicate the decomposition of anhydride groups of PMAO by DMAPA. Moreover, the bands in the spectra of hydrophobic QDs and PMAO-DMAPA are represented in the ZWL-QDs. These spectra confirmed that the surface ligands are attached to QDs.

Figure 3 shows the absorption and emission spectra of hydrophobic QDs and ZWL-QDs. The phase transfer causes the slight shift of both absorption and emission spectra due to solvent polarity changes or surface polymer physical/optical properties, but the overall shapes of spectra are retained. Moreover, the QY of native QDs in hexane is ~51 % (Huang

et al. 2013) and the QY of ZWL-QDs in water is ~49 %. The QY (or photoluminescent intensity) of ZWL-QDs is almost preserved during the phase transfer (inset images in Fig. 3). The surface modification method is good in preparing brighter QDs in aqueous solutions, compared to previously reported approaches, where QDs suffer 20 ~ 50 % loss of QY (or photoluminescent intensity) during phase transfer (Breus et al. 2009; Muro et al. 2010; Muro et al. 2012; Liu et al. 2013; Booth et al. 2013).

The hydrodynamic size of ZWL-QDs in water is around 7 ~ 10 nm, as shown in Fig. 4, which is comparable to many ZW-QDs using ligand exchange (Breus et al. 2009; Muro et al. 2010; Muro et al. 2012), but much smaller than that of PEG QDs (Uyeda et al. 2005; Yu et al. 2007; Mei et al. 2008). The measured zeta potential of ZWL-QDs in water is around 26 mV, which indicates a net positive surface charge of these QDs in water. The net positive surface charge is believed to be caused by the partial protonation of carboxyl groups on the QD surface in water.

The stability of the prepared ZWL-QDs was tested under various conditions. The photoluminescent intensity of these QDs (100 μ L, 50 nM) dispersed in PBS with pH 5–11, pure water, a 26 % NaCl solution, and human serum was monitored over 16 h at room temperature using fluorimetry. As shown in Fig. 5a, the photoluminescence intensity and hence stability of these QDs were maintained in solutions over a wide range of pH and in high salinity as well as in physiological media. Figure 5b shows images of several solutions of these ZWL-QDs under the same conditions after 16 h. The ZWL-QDs in glass tubes were illuminated with room light (top panel in Fig. 5b) and 405 nm UV light (bottom panel in Fig. 5b), respectively.

It is clear that the dispersions are stable at room temperature and no precipitates or significant luminescence intensity decreases were observed as evident from the homogeneous fluorescence of samples. Permanently charged groups on these ZWL-QD surfaces enhance the solubility of QDs under all tested conditions. In basic solutions, carboxylic groups are deprotonated to electrostatically stabilize the ZWL-QDs. In acidic solutions, carboxylic groups are partially protonated, but the permanent positive charges (quaternized amino groups) are dominant and the positive electrostatic repulsion keeps the ZWL-QD colloidal stable. In salt saturated solution (26 % NaCl), it is believed that both positive and negative charges on the ZWL-QD polymer surface bind water molecules via electrostatically induced hydration (positive charges on ZWL-QD polymer attract the OH⁻ component of water, and negative charges arrest the H⁺ component of water) (Liu et al. 2013). The hydration layer repulsion is less vulnerable to the ionic strength. The test also shows that these ZWL-QDs are stable in physiological media with complex matrix effects. The stability of these ZWL-QDs offers a great deal of flexibility for their applications in biological or biomedical experiments, where harsh conditions are ubiquitous. For instance, many cellular organelles are maintained under acidic conditions and rich in salts. Additionally, it should be noted that the samples as shown in Fig. 5b were prepared and observed over 21 days under 25 and 37 °C, respectively. No precipitates or significant photoluminescence intensity decreases were observed under these conditions. The ZWL-QDs were also tested to be stable in water at 4 °C over months. The long-term storage and associated stability under various conditions are under further investigation. Alternately, a

feasible approach includes drying the QDs, storing, and then re-suspending them in appropriate solutions before use. Please be noted that the photoluminescence intensity of the ZWL-QDs in PBS with pH 3 was observed to drop about 20 % during 16-hour monitoring, and thus the ZWL-QDs are not considered to be stable under pH 3 or a lower pH level. The reason for this instability under pH 3 is still under investigation. It is probably due to the coating degradation of amphiphilic polymer on QD surface in such an acidic condition.

The cytotoxicity of the ZWL-QDs was investigated on human primary glioblastoma cells (U-87 MG) and human embryonic kidney 293 cells (HEK-293). U-87 MG and HEK-293 represent tumor cells and normal cells, respectively. They may have different hardness and resistance to cytotoxic environments (Yu et al. 2007). Figure 6a shows the relative cell viability of both U-87MG and HEK-293 cells treated or incubated with 20 nM of the ZWL-QDs at different time points (untreated cells used as controls with 100 % cell viability). It can be seen that the average cell death is not more than 5 % compared to control, and the toxicity of the ZWL-QDs is insignificant. Figure 6b shows the relative cell viability for both cells after 72-hour incubation with the ZWL-QDs under different ZWL-QD concentrations. The maximum cell death or ZWL-QD toxicity occurs at 100 nM but less than 10 % compared to controls. Figure 6c–f shows the representative images of the live/dead cell staining assays on U-87 MG and HEK-239 for both the control and the sample after 72-hour incubation. Images in Figs. 6c, d (or e, f) are comparable in indicating the ratio of live cells (green spots) to dead cells (red spots). It is clear that the ZWL-QD toxicity to both cells is not significant in the tested dose range and time frame. In addition, the uptake of ZWL-QDs (20 nM) by both cells was observed in their incubation process. As shown in Fig. 7, when the incubation time increased from 4 to 16 h, more intense cytoplasmic fluorescence was observed. The enhanced staining suggests that the ZWL-QDs may have been internalized into cells but without significant toxicity. The minimal cytotoxicity and the cellular uptake of ZWL-QDs indicate the potential applications of these particles as drug vehicles for cell or tissue therapy.

Conclusion

In conclusion, a polymer encapsulation approach has been introduced to prepare ZWL-QDs. The fundamental physical/chemical/biological properties of ZWL-QDs have been characterized, which are important aspects to understand before applying ZWL-QD for further applications.

Due to intact of native hydrophobic ligands on QD surfaces, the ZWL-QDs nearly preserve the optical properties of hydrophobic QDs in organic solvents. Moreover, these ZWL-QDs have been shown to be compact in size and stable in solutions over a wide range of pH and with high salinity. Compared to other ligand exchange-based approaches, this polymer encapsulation method is simple and cost effective. The cytotoxicity and the cellular uptake of the ZWL-QDs were also studied on both human tumor cells and normal cells. It is found that the ZWL-QDs can be internalized by both different types of cells and present minimal cytotoxicity to them (in a concentration <100 nM).

Because of the unique properties of ZWL-QDs (small size, positively charged surface, ignorable cytotoxicity, etc.), it is believed that this study opens an avenue for the ZWL-QDs to be widely applied in biosensing, bioimaging, and drug delivery for cell or tissue therapy. For instance, the ZWL-QDs due to their positive charge surface can bind with negatively charged molecules through electrostatic interaction and can be used for drug delivery (Yezhelyev et al. 2008). They can also be substantially loaded more into the core of liposome due to their compact size and hydrophilic surface for more sensitive signal amplification in biosensing or imaging (Zhou et al. 2013; Al-Jamal and Kostarelos 2011). Besides, the developed surface modification approach can be applied for the phase transfer of many other hydrophobic nanoparticles such as oleic acid-coated Fe₃O₄ magnetic particles. The future work of this study will focus on the further development (e.g., surface charge tuning to make ZWL-QDs negatively charged for electrostatically positive molecule absorption) and bio-applications of the proposed surface modification method.

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Fig. 1.
Synthesis of ZWL-QDs using a polymer encapsulation approach

Fig. 2.
FT-IR spectra of materials collected during each synthetic step of ZWL-QDs

Fig. 3. UV-Vis absorption and photoluminescence (PL) spectra of hydrophobic QDs in chloroform and ZWL-QDs in water (the inset images demonstrate the phase transfer of QDs from the bottom chloroform layer to the top water layer before and after surface modification)

Fig. 4.
The hydrodynamic size of prepared ZWL-QDs in water measured by dynamic light scattering

Fig. 5.

a Photoluminescence (PL) intensity of the prepared ZWL-QDs (50 nM) dispersed in PBS with pH 5–11, pure water, a 26 % NaCl solution, and human serum over 16 h under room temperature; **b** Photograph of the ZWL-QDs after 16 h under room temperature in PBS with pH 5–11, pure water, and a 26 % NaCl solution. The top panel image shows ZWL-QDs in different solutions under room light, and the bottom panel is the PL image of ZWL-QDs under 405 nm UV light. Images of ZWL-QDs in human serum are not shown due to the natural turbidity of serum

Fig. 6. Relative cell viability of U-87 MG and HEK-293 cells treated with 20 nM ZWL-QDs at different time points (a); Relative cell viability of U-87 MG and HEK-293 cells incubated with ZWL-QDs for 72 h at different ZWL-QD concentrations (b); Representative images of live/dead cell staining assays on HEK-293 (c and d) and U-87 MG (e and f) for both the control and the sample after 72-hour incubation

Fig. 7. HEK-293 (a–b) and U-87 MG (c–d) were imaged using both a bright field and a fluorescence filter (ex 485 nm/em 655 nm). ZWL-QDs (20 nM) were incubated with cells for 4 and 16 h (a vs. b and c vs. d). Staining shows punctate patterns that are enhanced by longer incubation times, suggesting that the ZWL-QDs may have been taken into the cell by micropinocytosis and are concentrated in membrane-bound vesicles